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Breast cancer prevention—clinical trials strategies involving aromatase inhibitors☆

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Abstract

Estrogens and their metabolites have been implicated in both the initiation and the prevention of breast cancer. The reduction in breast cancer incidence seen in the tamoxifen arms of the four prospective trials to date has established the proof of principle that antagonizing estrogen is a potential means of reducing breast cancer risk. However, the areas to improve on these results include: (a) enhanced efficacy, (b) reduction in the incidence of receptor-negative tumors, (c) improved overall and endocrinological side effects, and (d) improved function on end-organs other than the breast. The aromatase inhibitors offer the potential to achieve these goals in part in the following ways: (a) greater reduction in risk of disease as evidenced by superior efficacy in advanced breast cancer and by inhibition of both initiation and promotion of breast cancer, (b) reduction in receptor-negative tumors by synergy with COX-2 inhibitors resulting in growth factor inhibition, anti-angiogenesis and inhibition of tumor-associated aromatase expression, (c) fewer vasomotor and urogenital abnormalities, and (d) reduced thromboembolism and cardiovascular complications and satisfactory effects on bone metabolism. Important differences may exist between non-steroidal reversible inhibitors and steroidal irreversible inactivators in particular related to the androgenic/anabolic effects of the steroidal inactivators. Pilot studies of aromatase inhibitors described elsewhere in this session have begun in healthy women with dense mammography, or a high-risk genetic and/or histocytopathologic profile, to determine potential efficacy, as well as effects on end-organ function. A number of phase three trials with aromatase inhibitors are also underway or in planning. Among these are the BRCA 1 and 2 study of exemestane versus placebo in unaffected postmenopausal carriers, the International Breast Intervention Study 2 (IBIS 2) of anastrozole versus placebo in women with a high-risk profile, and the National Cancer Institute of Canada's Clinical Trial Group (NCIC CTG) study of exemestane with or without celecoxib versus placebo in women at risk of the disease. For premenopausal women, combination strategies of gonadotrophin agonists and aromatase inhibitors are being investigated. The potential of using low doses of aromatase inhibitors to lower "in breast" estrogen levels without unduly perturbing plasma concentrations is also being explored. The potential of the aromatase gene functioning as an oncogene within the breast may be tied to breast density which in turn may represent both a selection tool for elevated risk and an intermediate marker of prevention. The strong link between postmenopausal estrogen levels and breast cancer risk suggests the possibility that plasma estrogen levels may be a useful intermediate marker of prevention. The aromatase inhibitors offer us the first ever tool to render women virtually free of estrogen and are potentially an exciting tool for the prevention of breast cancer.

Keywords: Aromatase inhibitors; Breast cancer; Prevention; Clinical trials designs

1. Introduction

A modest impact on breast cancer mortality has been achieved by the implementation of improved screening techniques together with improved surgical, radiation and systemic treatments. In particular, adjuvant hormone therapy has reduced mortality significantly but there is a substantial unmet medical need to either improve the outcome of patients already diagnosed with invasive breast cancer or alternatively to reduce the occurrence or progression of preinvasive breast lesions in order to prevent invasive cancer and thereby to reduce mortality. The precise genetic steps required for epithelial cells to transform from normal to breast carcinoma are ill understood, particularly in the early stages of the disease when many common chromosomal losses have been identified but the precise molecular targets remain elusive. However, by the time ductal carcinoma in situ has evolved, estrogen receptor, COX-2, c-erb B-2, cyclin and p53 overexpression are noted and aneuploidy is frequent. This suggests that at the stage of DCIS, or even earlier, interruption of these pathways may delay or avoid progression

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to invasive lesions or "prevention". The role of antagonizing estrogen, and in particular with inhibition of the estrogen synthetase (aromatase) enzyme complex, is outlined in this article and the potential for additive or synergistic effects between aromatase inhibitors and other biological agents is discussed.

2. Estrogen and breast cancer risk

There is abundant in vitro, in vivo and human data to suggest a pivotal role for estrogens in both the initiation and progression of breast cancer. Genotoxic reactive metabolites of estrogen, in particular the catechol estrogens 2- or 4-hydroxyestradiol are considered weak estrogen-independent initiators of cancer [1]. In addition, promotion of known receptor-positive hormone-dependent breast tumors in experimental animals, as well as hyperplastic breast lesions resulting from breast-specific aromatase gene upregulation in transgenic mice, suggest a powerful estrogen receptor-positive promotion of breast tumors [2]. Data from the use of estrogens and hormone replacement therapy indicate that exogenous estrogens are also strongly correlated with breast cancer risk [3]. In addition, several meta-analyses of prospective studies of estradiol and breast cancer risk in postmenopausal women have tightly correlated plasma hormone levels, plasma estradiol, free estradiol, estrone and testosterone levels with breast cancer risk [4]. Antagonizing estrogen would therefore seem a logical means of lowering this risk. In premenopausal women, circulating estrogen levels come predominantly from ovarian production of estrogens. In contrast, in postmenopausal women, peripheral aromatization of androgens to estrogens is a principal source of plasma estrogen. Of note, however, is the observation that intra-breast estrogen production in postmenopausal women increases substantially, resulting in comparable concentrations of estrogen in the breasts of both pre- and postmenopausal women despite peripheral plasma levels being markedly lower in the postmenopausal setting [5]. This has in part been shown to be due to breast fibroblast aromatization and provides an additional target of antagonizing estrogen and possibly preventing breast cancer.

3. Antagonizing estrogen as a breast cancer prevention strategy

Significant proof of principle that antagonizing estrogen in humans will lead to reduction in breast cancer incidence has been obtained from four large prospective placebo-controlled clinical trials in women at increased risk for breast cancer. These trials include the large NSABP P1 study, International Breast Intervention Study 1 (IBIS 1), the Italian Breast Cancer Prevention Study, and the Royal Marsden Hospital Breast Cancer Prevention Trial. All four of these trials have shown a statistically significant reduction in the short-term occurrence of breast cancer in women treated with tamoxifen as opposed to placebo. The meta-analysis of the data from these four studies together with the Oxford overview meta-analysis of contralateral breast cancer reduction from adjuvant tamoxifen reveals a net 38% reduction in invasive and preinvasive cancer [6]. In addition, a significant reduction in estrogen-related benign breast diseases has subsequently been shown by the NSABP including: reduction in adenosis, cysts, duct ectasia, fibrocystic disease, atypical hyperplasia, and an overall reduction in the need for clinical biopsies [7]. These proof of principle data imply that an interruption in the progression from preinvasive to invasive breast lesions is achievable through antiestrogenic means. Additional evidence for a steep dose-related curve of plasma estrogen levels with breast cancer risk has been obtained through the context of the multiple outcome raloxifene study in postmenopausal women with osteoporosis (MORE study). As shown in Fig. 1, reduction in the risk of breast cancer within the context of the study was seen most impressively in women with the highest tertile of postmenopausal serum estradiol level [8]. All of this suggests that inhibiting aromatase and reducing postmenopausal serum and intra-breast estrogen levels may result in a reduction in breast cancer risk. In the NSABP P1 study and in the ongoing STAR trial (tamoxifen versus raloxifene for 5 years in postmenopausal women) a Gail score greater than 1.66 has been a principal entry criterion. Increasingly, additional means of selecting postmenopausal women at high risk of breast cancer have been sought. Clinical markers of cumulative lifetime estrogen exposure have been identified as potential ways of achieving this, including elevated bone mineral density and plasma estrogen levels (as above). In addition, breast density on screening mammography in healthy women has also been shown to be a marker of breast cancer risk and affords another potential selection tool for risk identification. The most definitive assessment of premalignant lesions comes

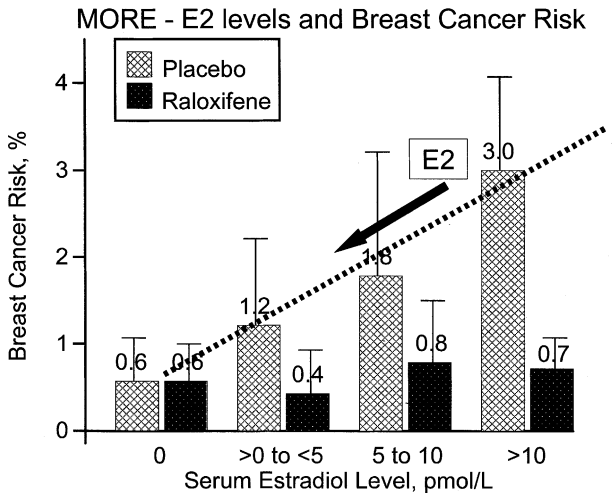

Fig. 1. Baseline serum estradiol levels and relative risk and risk reduction in the multiple outcomes of raloxifene (MORE) study.

from cyto- or histopathologic evaluation of breast cells and to this end various techniques are being applied to characterize particular preinvasive lesions. These include fine needle aspiration targeted in a blind fashion in the upper outer quadrant of the breast, or to an area of breast density, or systematically in a periareolar fashion. In addition, nipple aspirate fluid has been obtained via ductal lavage of the breast. These research tools are being used in ongoing breast cancer prevention pilot studies of aromatase inhibitors.

4. Aromatase inhibitors and breast cancer prevention

As mentioned above, an alternative to tamoxifen for antagonizing estrogen's ability to either initiate or promote breast cancer is afforded by inhibition of aromatase and reduction of peripheral and intra-breast estrogen levels. Aromatase inhibitors are already in clinical use in breast cancer patients with receptor-positive postmenopausal disease. Two classes are represented, the reversible nonsteroidal imidozoles represented by anastrozole and letrozole and the irreversible steroidal inhibitor exemestane. The irreversible inhibition of the enzyme complex together with possible additional anticancer activity through the androgen receptor pathway and potential androgenic/anabolic effect on end organs may confer a superior therapeutic index on the steroidal inhibitor as compared with the nonsteroidals. Exemestane and its major rat and human metabolite 17-hydroexemestane have been studied in a model of androgenicity in immature castrated rats. Both the parent compound and its metabolite have been shown to have androgenic and specifically anabolic effects in this model. In addition, antiproliferative effects of exemestane on hormone receptor-positive breast cancer cells in vitro are partially reversible with the anti-androgen receptor agent flutamide indicating that in addition to aromatase inhibition exemestane and its metabolite may exert a therapeutic benefit through this second pathway. In 10-month-old cycling Sprague–Dawley rats, exemestane's effects on bone and lipid metabolism have been recently demonstrated and contrasted with the nonsteroidal inhibitor letrozole. In this model, exemestane both preserves femoral bone mineral density and protects from adverse cholesterol changes in a dose-dependent manner, as does the principal metabolite 17-hydroexemestane. These changes are not observed with letrozole when coadministered with oophorectomy in this model.

At least eight international adjuvant breast cancer trials are ongoing in postmenopausal women with receptor-positive

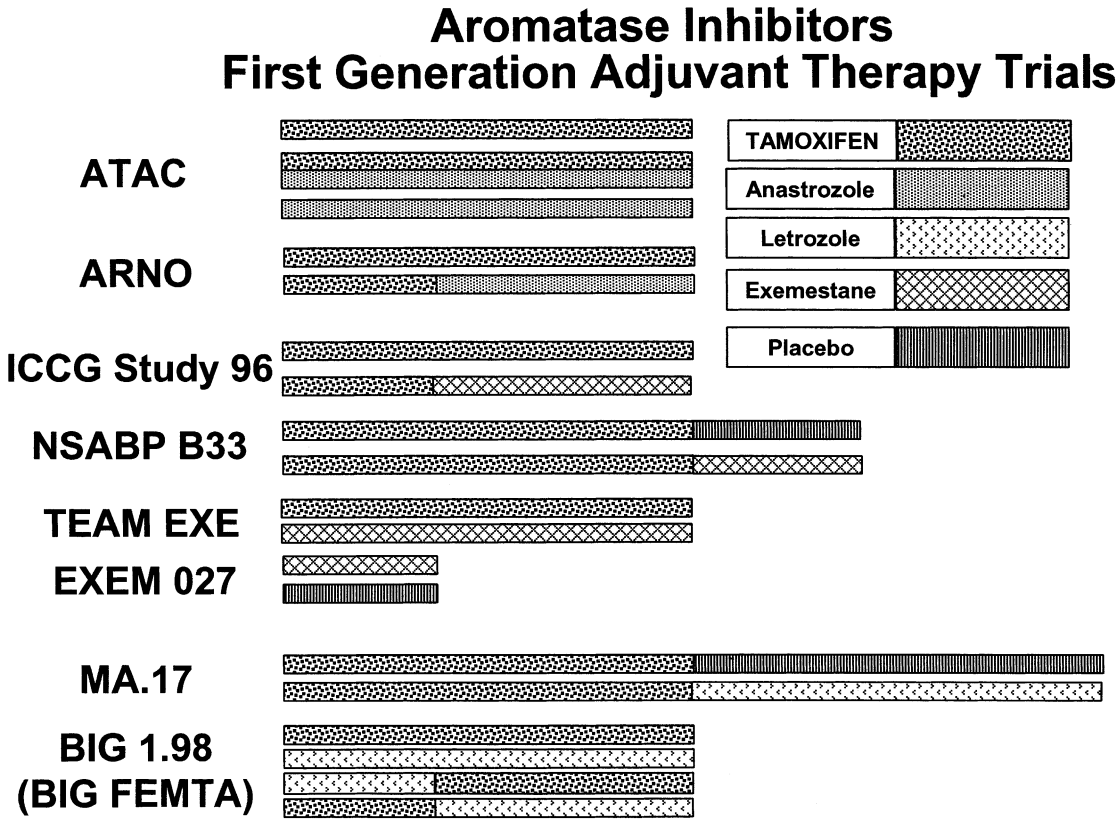

Fig. 2. The designs of ongoing adjuvant trials of aromatase inhibitors in early stage postmenopausal receptor-positive breast cancer. ATAC: Arimidex vs. Tamoxifen Alone or in Combination; ARNO: arimidex–nolvadex; ICCG: International Collaborative Cancer Group—tamoxifen vs. exemestane for 2–3 years of tamoxifen; NSABP B33: National Surgical Adjuvant Breast and Bowel Project B33; TEAM EXE: exemestane vs. tamoxifen for 5 years; EXEM 027: comparing exemestane to placebo for 2 years; MA.17: letrozole vs. placebo in women with primary breast cancer completing 5 or more years of adjuvant tamoxifen; BIG 1.98 (BIG FEMTA): tamoxifen (5 years) vs. letrozole (2 years) followed by tamoxifen (3 years).

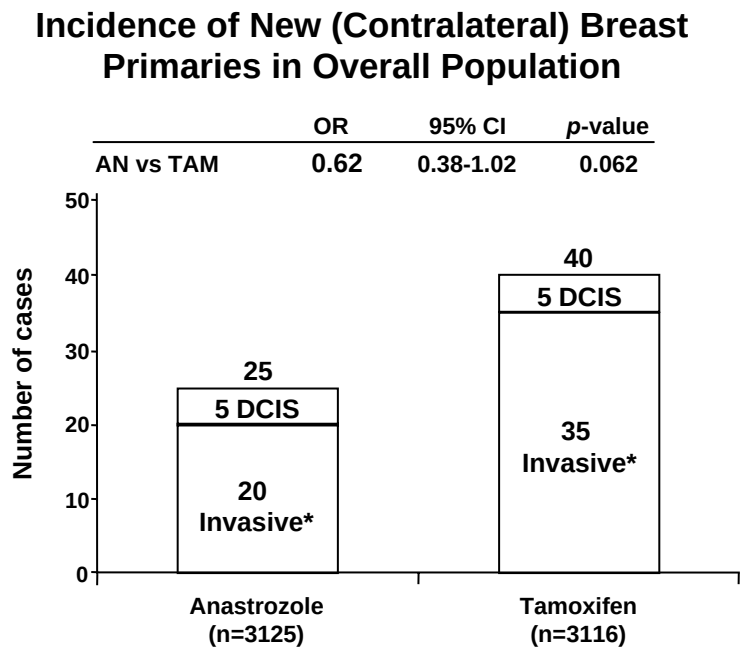

Fig. 3. Disease-free survival in the ongoing ATAC trial.

breast cancer. All of these studies will provide contralateral breast cancer incidence and afford a view of potential preventative effects of this class of agents. These studies are shown in Fig. 2. The first trial to report is the Arimidex versus Tamoxifen Alone or in Combination (ATAC) study, and reduction in contralateral breast cancer incidence from tamoxifen, tamoxifen plus anastrozole or anastrozole alone is shown in Fig. 3 [9]. An excess of clinical fractures and an increase in musculoskeletal symptoms occurred in the anastrozole-treated arm of the study. Other known side effects of tamoxifen, including vaginal bleeding, endometrial cancer, and thromboembolism were seen significantly less often in the anastrozole-treated patients. These data strongly suggest an equivalent or greater potential reduction in breast cancer risk from the aromatase inhibitor and a potentially important improvement in therapeutic index with the avoidance of very serious side effects. The steroidal inhibitor exemestane may afford a superior profile to anastrozole as a chemopreventative, both in terms of efficacy against breast cancer as mentioned above, by a dual mechanism of action, and in addition the possibility of its beneficial effects on important end organ functions such as bone and lipid metabolism.

5. Ongoing chemoprevention pilots and definitive phase III breast cancer prevention trials

The National Cancer Institute of Canada's Clinical Trial Group (NCIC CTG) is conducting a pilot feasibility study of letrozole in postmenopausal women with breast density on a screening mammogram either in healthy women or those with prior receptor-positive breast cancer and greater than 6 months from completing adjuvant tamoxifen. The primary end point of this trial is reduction in mammographic density and particular end points include bone marrow density, plasma lipid levels and general toxicities. In selective women, serial core biopsies or fine needle aspiration biopsies are also being obtained from areas of density within the breast with a view to cytopathologic evaluation (Fig. 4). In Italy, a randomized phase III breast cancer prevention trial is underway in unaffected postmenopausal women who have known mutations for the BRAC1 or 2

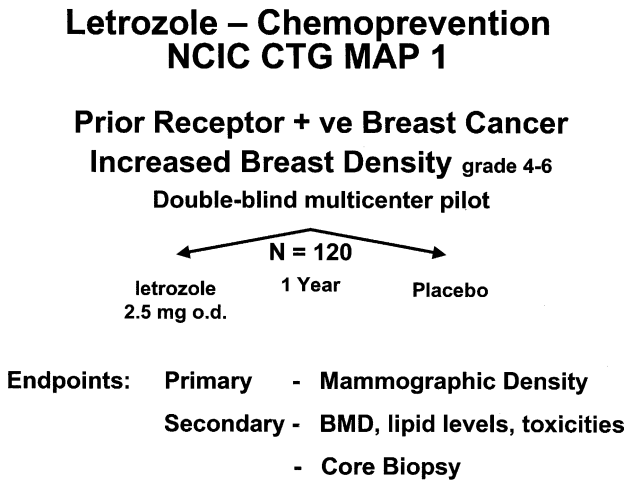

Fig. 4. NCIC CTG trial of letrozole vs. placebo in postmenopausal women with increased density on mammography.

gene. This trial randomizes women to exemestane 25 mg daily versus placebo for a period of 3 years. The primary end point is incidence of breast cancer. A proposal to include premenopausal women in this trial randomizing them to a gonadotrophin releasing hormone (GnRH) agent plus either exemestane or placebo is under discussion. Following the results of the ATAC adjuvant breast cancer trial mentioned above and publication of the recent IBIS 1 study [10], the IBIS consortium is undertaking a randomized phase III (IBIS 2) study of anastrozole versus placebo in postmenopausal women at increased risk of breast cancer as defined by criteria similar to the IBIS 1 study. Multiple secondary end points are being investigated within the confines of this trial including disease-free, overall and cause specific mortality, as well as bone mineral density.

6. Inhibitors of COX-2 as potential aromatase inhibition and synergy with aromatase inhibitors

The cyclooxygenase-2 pathway is an inducible and upregulated pathway in both preinvasive and invasive cancers. COX-2 overexpression can lead to pleiotropic mechanisms of carcinogenesis including angiogenesis, cell growth and invasion, tumor-associated inflammation, inhibition of apoptosis and an association with c-erb-2 upregulation and aromatase induction [11]. COX-2 inhibitors such as celecoxib and rofecoxib therefore afford the potential for breast cancer reduction, both in terms of incidence and tumor burden. In addition to antagonizing, the COX-2-dependent targets mentioned above, COX-independent targets have also been described for this class of drugs. Celecoxib in a dose-dependent manner inhibits both the incidence (prevention) and burden (treatment) of DMBA-induced rat mammary carcinomas which is an animal model of receptor-positive breast cancer. In addition, there is significant synergy between exemestane and celecoxib in the DMBA-induced model, as shown in Fig. 5 [12]. Based on these findings, the NCIC CTG is leading a North American intergroup adjuvant breast cancer trial (the MA.27 study) in which 6800 newly diagnosed postmenopausal women with receptor-positive breast cancer will be randomized to anastrozole versus exemestane for 5 years with or without celecoxib for 3 years. The primary end points include disease-free and overall survival but contralateral breast cancer reduction is an important secondary end point and should provide preventative data regarding these agents. Table 1 outlines the possibility of inhibiting both peripheral and intra-breast aromatase with either full-dose aromatase inhibitors or full-dose aromatase inhibitor with add-back physiological levels of estrogen or low-dose aromatase inhibition or COX-2 therapy as monotherapy or in conjunction with an aromatase inhibitor. Because both tamoxifen and raloxifene have been shown to reduce only ER+ breast cancer, and celecoxib, specifically in addition to its pleiotropic antitumor mechanisms and its effect in the hormone receptor-positive animal model, also causes a dose-dependent reduction in estrogen receptor-negative cell lines [13], the NCIC CTG is launching a randomized phase III trial in postmenopausal woman at increased risk of breast cancer randomizing between placebo, exemestane and exemestane with celecoxib. The rationale is potent efficacy of breast cancer reduction by exemestane, and improved end organ profile from this compound as compared to nonsteroidal inhibitors and the potential to synergize with celecoxib in breast cancer reduction thereby hopefully reducing both ER +ve as well as ER −ve tumors with a long-term goal of reducing mortality (Fig. 6).

Table 1
Potential breast cancer prevention strategies: aromatase inhibition

	Peripheral aromatase	Intra-breast aromatase
Full dose AI	↓↓	↓↓↓
Full dose AI + add back E2	Normal	↓↓
Low-dose AI	Normal	↓↓
COX-2 inhibitor	Normal	↓
COX-2 inhibitor + AI	↓↓	↓↓↓

It is of interest to note that the combination arm in the ATAC trial of anastrozole plus tamoxifen may result in a worse outcome than with either agent alone. The putative

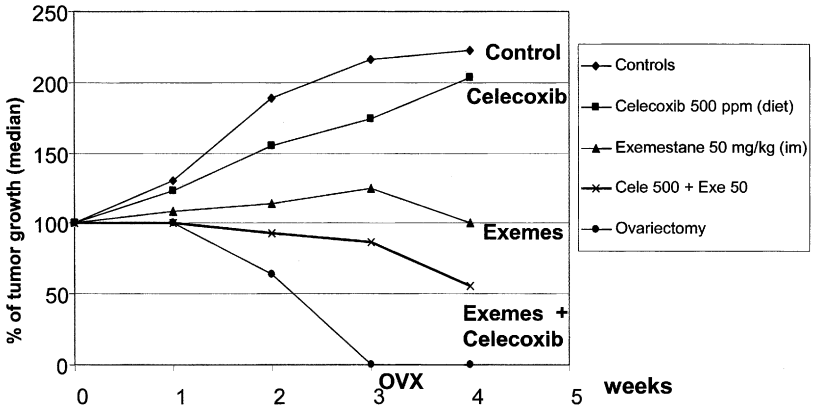

Fig. 5. Celecoxib enhances exemestane tumor growth inhibition in DMBA-induced breast cancer ER +ve model.

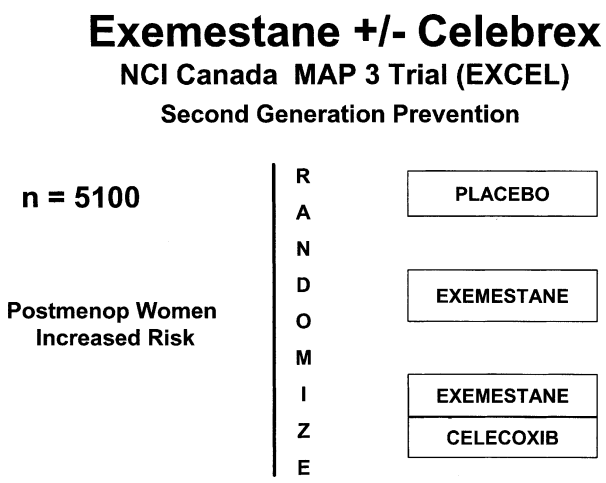

Fig. 6. NCIC CTG: exemestane +/− celecoxib vs. placebo in postmenopausal women at increased risk of breast cancer.

explanation for this is enhanced estrogen agonist effect of tamoxifen in the presence of depleted ambient estrogen levels. This hypothesis was outlined by Morello et al. [14] and has been shown to possibly be less so for SERMs which are inherently less agonistic. Newer generation SERMs may perform better in combination with an aromatase inhibitor and the opportunity to explore a total estrogen blockade, as initially conceived for the combination arm of the ATAC trial, may yet be an achievable goal. Clinical trials are under way in the metastatic setting in this regard.

7. Conclusion and future directions

Antagonizing estrogen appears to be a cornerstone strategy for reducing the incidence of breast cancer. Tamoxifen has produced the proof of principle in this regard but has an unwanted therapeutic index creating significant toxicities including endometrial cancer and thromboembolism in healthy women. The American Society of Clinical Oncologists Technology Assessment Group reviewing potential application of tamoxifen as a breast cancer preventative has determined that there is no apparent net health benefit to women taking this drug because of its unwanted toxicities and therefore advises placebo-controlled trials for future phase III breast cancer prevention trials. The aromatase inhibitors afford a potentially superior strategy to tamoxifen for preventing hormone receptor-positive cancers. In particular, they appear more efficacious than tamoxifen in the metastatic, neoadjuvant, and probably adjuvant setting. Their potential to inhibit both initiation and promotion of breast cancer may be an added advantage. The steroidal inhibitor exemestane may in turn be superior to the nonsteroidal inhibitors both in terms of its efficacy, having a potential dual mechanism action through the androgen receptor pathway and by virtue of an enhanced therapeutic index through mild androgen effects on bone and lipid metabolism. The IBIS Consortium of Investigators is launching a definitive phase III placebo-controlled study of anastrozole in postmenopausal women at risk for breast cancer and the North American Consortium of Women's Health Investigators and Oncologists are launching, through the NCIC CTG, a placebo-controlled trial of exemestane. This latter trial, however, additionally includes celecoxib, an inhibitor of the COX-2 pathway, which is an approved cancer prevention drug (for familial polyposis of the colon) and apparently synergizes significantly with exemestane in preclinical animal models. Due to its pleiotropic mechanisms of action and its inhibitory effect on receptor-negative breast cancer cells in vitro, it may also achieve a reduction in estrogen receptor −ve cancer which disproportionately contributes to overall breast cancer mortality. Finally, antagonizing estrogen but additionally blocking other potential pathways of breast cancer progression may be an additional way forward in reducing both the incidence and mortality of breast cancer.

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